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Decreased responsiveness of the aortae of hypertensive rats to acetylcholine, histamine and noradrenaline

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1 The responses to noradrenaline (NA) of the aortae of various hypertensive rats, namely the spontaneously hypertensive rat (SHR), the low blood pressure SHR (LBP-SHR), and the left renal artery stenosed LBP-SHR (LRAS-LBR-SHR), were compared to those of the normotensive Wistar-Kyoto rats (WKY).

2 The aortae of the hypertensive rats were significantly more responsive ($P < 0.05$) to 10^{-8} M NA. However, the reverse was true for higher doses of NA. The ED_{50} values for the aortae of WKY, LBP-SHR, SHR and LRAS-LBP-SHR were 20, 8.5, 7.8 and 8 nM respectively.

3 The NA-contracted aortae of the LRAS-LBP-SHR were significantly less responsive ($P < 0.05$) to the relaxant action of histamine and acetylcholine (ACh) compared to those of the WKY. This observation was not made in the aortae of the LBP-SHR. The maximal relaxation (% of the maximal contraction induced by 10^{-8} M NA) observed in the aortae of WKY, LBP-SHR and LRAS-LBP-SHR were, respectively, 72 ± 2, 66 ± 6, 39 ± 7 for ACh and 50 ± 3, 36 ± 4, 27 ± 3 for histamine.

4 In aortae where the endothelium had been removed by collagenase treatment, histamine induced a dose-related contraction. The rank order of this dose-related contraction was WKY > LBP-SHR > SHR > LRAS-LBP-SHR with the corresponding maximal tension (g) 0.89 ± 0.04, 0.59 ± 0.04, 0.36 ± 0.04, 0.19 ± 0.05.

5 The results suggested that elevation of blood pressure above the normal (due either to intrinsic or extrinsic factors), as seen in SHR and LRAS-LBP-SHR, results in a decreased response of the aortae to ACh and histamine. This effect was seen in both the endothelium mediated relaxation and the non-endothelium mediated contraction.

Introduction

The blood vessels of spontaneously hypertensive rats (SHR) have been found to differ from those of normotensive Wistar-Kyoto rats (WKY) in their relaxation responses to acetylcholine (ACh), adenosine and isoprenaline (Triner *et al.*, 1975; Cohen & Berkowitz, 1976; Shibata & Cheng, 1978). In a recent study, we also showed that the noradrenaline (NA)-contracted aortae of renal hypertensive rats and SHR were hyporesponsive to the relaxant action of histamine, but differed significantly in their relaxation response to ACh, i.e. the aortae of the former animals were hyporesponsive whilst the aortae of the latter animals were not (Sim & Chua, 1985). In an attempt to identify which of the endothelium, smooth muscle or genetic factors was a possible cause for this difference, we studied: (1) the relaxant actions of histamine and ACh on NA-contracted aortae of SHR whose blood pressures were below 150 mmHg (LBP-SHR), on the same type of animals whose left renal arteries had been stenosed for a period of 4 weeks (LRAS-LBP-SHR), and on normotensive WKY; (2) the action of NA on the aortae of the same 3 types of animal and SHR whose blood pressures were above 150 mmHg; (3) the action of histamine on the collagenase-treated aortae of the same types of animal as in (2).

Methods

The rats used were 4–6 months old and of the following strains, systolic blood pressure and heart rate: (i) normotensive WKY, 122 ± 9 mmHg, 273 ± 15 beats min^{-1} ($n = 31$); (ii) SHR, 180 ± 20 mmHg, 337 ± 14 beats min^{-1} ($n = 21$); (iii) LBP-SHR, 133 ± 12 mmHg, 287 ± 84 beats min^{-1} ($n = 32$); (iv) LRAS-LBP-SHR, 230 ± 50 mmHg, 328 ± 19 beats min^{-1} ($n = 36$). SHR stock was obtained from Professor Y. Yamori, Shimane Medical University, Japan. The rats were bred in the depart-

ment by sibling mating, of which 10–15% of the offspring had a blood pressure of less than 150 mmHg (LBP-SHR). Systolic blood pressure and heart rate were measured in the conscious rats by tail plethysmography. The operation to stenose the left renal artery was carried out under methoxyhexital sodium anaesthesia (4 mg 100 g^{-1}, i.p.) A flank incision was made on the left side of the animal and a silver clip with a 0.2 mm clearance between the two arms was placed over the renal artery. After the stenosis operation, the blood pressure of the LRAS-LBP-SHR rose rapidly in the first 10 days and reached a maximal value within a month.

Rats were killed by a blow on the head and two thoracic aortic rings of 3 mm per rat were prepared and fixed isometrically in a 10 ml muscle chamber containing Krebs-Ringer bicarbonate solution (composition, mM: NaCl 135, KCl 5, $NaHCO_3$ 20, glucose 10, $CaCl_2$ 2.5, $MgSO_4$ 7 H_2O, 1.3, KH_2PO_4 1.2, EDTA 0.026) kept at 37°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Each ring was stretched with a tension of 1 g for 2 h. Care was exercised to preserve the integrity of the endothelium throughout the preparation. The first ring was then contracted with 10^{-8} M NA and, 8 min later (at maximal contraction), increasing doses of ACh were cumulatively added, at 2 min intervals, to produce a graded relaxation. The drugs were then washed out and, after a 60 min rest period, the experiment was repeated with histamine instead of ACh. For the second ring, histamine was used to produce relaxation before ACh.

The second part of the study was carried out to investigate the responses of the aortae to increasing doses (10^{-9}–10^{-5} M) of NA. After a period of 6 min to allow for maximal contraction, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M NA were added cumulatively with an interval of 6 min between each addition. The tension developed with the cumulative doses of NA was expressed in g.

The third part of the study was carried out with the collagenase-treated aortae. Cannulated aortae were filled with 0.2% collagenase Type 1 (Sigma) and incubated at 37°C in Krebs-Ringer solution for 20 min. After rinsing, a section of each aorta was then set aside for histological examination and two 3 mm rings were similarly prepared and contracted with 10^{-8} M NA. Failure of 10^{-6} M ACh to relax the NA-contracted aortae was taken to indicate that the endothelium had been removed. This was confirmed histologically by the paraffin wax method where the cut sections were stained with haematoxylin and eosin. Each ring was then contracted with cumulative doses of histamine (10^{-6}–10^{-3} M) as described for NA. There was no change in pH of the Krebs-Ringer bicarbonate solution in the 10 ml muscle chamber after a maximal volume of 100 µl of the various drug solutions had been added.

Drugs

NA (arterenol, Sigma) was dissolved in 1% ascorbic acid. Acetylcholine chloride (Sigma) was prepared as a stock solution of 1 M in 0.5 M NaH_2PO_4. Histamine (Sigma) was prepared as a stock solution of 1 M in distilled water. Collagenase Type I (Sigma) was prepared in phosphate buffered saline.

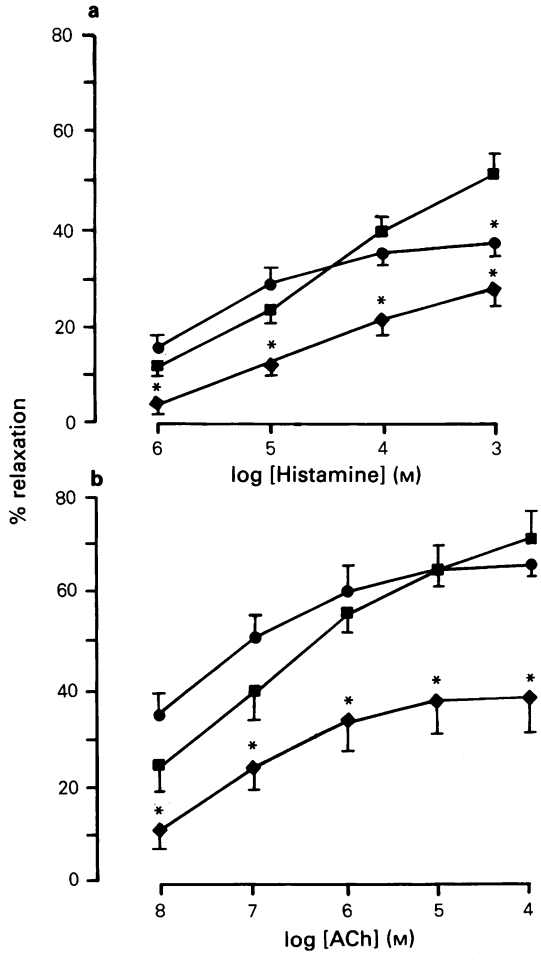

Figure 1 Concentration-response curves for the relaxation of the noradrenaline-contracted aortae of WKY (■), LBP-SHR (●), and LRAS-LBP-SHR (◆) to various doses of histamine (a) and acetylcholine (b). Responses are expressed as % relaxation of the maximal contraction induced by 10^{-8} M noradrenaline. *Indicates significant difference ($P < 0.05$) between WKY and the other animals. Each point represents the mean and vertical lines show s.e.mean; $n = 10$–13.

Statistical analysis

The data are expressed as mean ± s.e.mean. Tests of significance were performed by use of 2-way analysis of variance and Student's t test. P values of less than 0.05 were considered significant.

Results

Relaxation responses

Figure 1 shows that histamine and ACh relaxed the NA-contracted aortae in a dose-dependent manner with ACh being a more potent vasodilator of the blood vessel. The responses of the aortae of WKY and LBP-SHR to the two vasodilators (except to 10^{-3} M histamine) were not significantly different ($P > 0.05$) from each other. However, the aortae of LRAS-LBP-SHR, compared to the aortae of WKY, were significantly less responsive ($P < 0.05$) to doses of histamine and ACh.

Responses to noradrenaline

Figure 2 shows that the aortae of the 4 types of rat contracted to increasing doses of NA (10^{-8}–10^{-5} M) in a dose-dependent manner; 10^{-9} M NA produced no appreciable contraction in any of the aortae tested. At 10^{-8} M, the responses (742 ± 31 mg) of the aortae of the WKY were significantly less ($P < 0.05$) than those of the LBP-SHR, SHR and LRAS-LBP-SHR which were, respectively, 879 ± 44, 946 ± 55, 832 ± 59 mg. However, at higher doses of NA (10^{-7}–10^{-5} M) the aortae of the hypertensive rats were significantly less responsive ($P < 0.05$), compared to those of the normotensive WKY. Of the hypertensive rats studied, the aortae of the LRAS-LBP-SHR were found to be the least responsive ($P < 0.05$) to all the doses (except 10^{-8} M) of NA tested, whilst the aortae of the LBP-SHR and SHR responded similarly ($P > 0.05$), except to 10^{-8} M NA. The maximal tension (g) of the responses developed in the aortae of WKY, LBP-SHR, SHR and LRAS-LBP-SHR was 1.8 ± 0.09, 1.5 ± 0.07, 1.5 ± 0.05 and 1.3 ± 0.08, respectively.

Responses to histamine

Figure 3 shows that all the aortae with the endothelium removed contracted in a dose-dependent manner to histamine. The aortae of hypertensive rats were found to be significantly less responsive ($P < 0.05$), to all six doses of histamine used, compared to those of normotensive WKY. Of the hypertensive rats, the

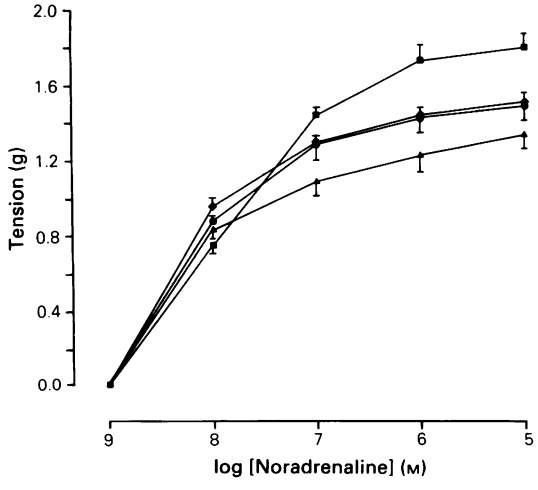

Figure 2 Concentration-response curves for the contraction (developed tension, g) of the aortae of WKY (■), LBP-SHR (●), SHR (◆) and LRAS-LBP-SHR (▲) to 10^{-9}–10^{-5} M noradrenaline. The tension of the responses developed in all the aortae of hypertensive animals was significantly lower than that obtained for WKY for all the concentrations tested ($P < 0.05$), except 10^{-8} M NA. Each point represents the mean and vertical lines show s.e.mean; $n = 13$.

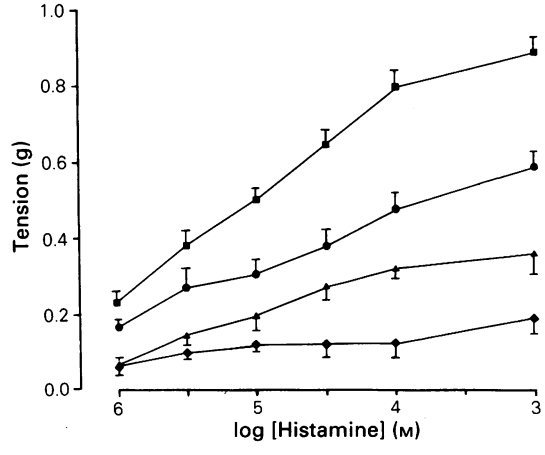

Figure 3 Concentration-response curves for the contraction (developed tension, g) of the collagenase-treated aortae of WKY (■), LBP-SHR (●), SHR (▲) and LRAS-LBP-SHR (◆) to various doses of histamine. The tension of the responses developed in all the hypertensive animals was significantly lower than that obtained for WKY for all the concentrations tested ($P < 0.05$). Each point represents the mean and vertical lines show s.e.mean; $n = 7$–10.

rank order of responsiveness to histamine with respect to tension developed was LBP-SHR>SHR->LRAS-LBP-SHR. The maximal tension (g) of the responses developed in aortae of WKY, LBP-SHR, SHR and LRAS-LBP-SHR was 0.89 ± 0.04, 0.59 ± 0.04, 0.36 ± 0.04 and 0.19 ± 0.05, respectively. The corresponding ED_{50}s for WKY, LBP-SHR, SHR and LRAS-LBP-SHR were, 7.5, 8.5, 8.5 and 4 μM respectively.

Discussion

The results indicate that the decreases in the responses of aortae of hypertensive rats to various vasoactive agents tend to be related to blood pressure. In the LRAS-LBP-SHR, where the blood pressure rose rapidly to a mean of 230 mmHg, the relaxant responses of the aortae to histamine and ACh were significantly smaller ($P<0.05$) than those exhibited by the control LBP-SHR and WKY. However in an earlier study (Sim & Chua, 1985), we found that the aortae of SHR and stroke prone SHR, compared to the aortae of WKY, were not significantly different in their responses to ACh, despite the fact that the former two strains of animals were hypertensive. Thus, it is possible that, besides the magnitude of the blood pressure, the onset and cause of hypertension can also affect the sensitivity of the aortae to ACh. This hypothesis is supported by the rapid development of hypertension in our LRAS-LBP-SHR and the reported slower rise of blood pressure in SHR (Lias *et al.*, 1977), and the renal-induced hypertension in the LRAS-LBP-SHR, which is probably different from the underlying causes of hypertension in the SHR. Since the LRAS-LBP-SHR, LBP-SHR and SHR are genetically of the same strain, the possibility of genetic factors contributing to this difference is minimal.

Konishi & Su (1983) found that the intact aortae of SHR showed a decreased responsiveness to various doses of NA (10^{-8}–10^{-5} M) compared to those of the WKY. We also observed a similar but significant ($P<0.05$) decreased responsiveness to 10^{-7}–10^{-5} M NA (see Figure 2). The different magnitude of this decrease is possibly due to the fact that the blood pressure of the WKY used by Konishi & Su was in the blood pressure range of our LBP-SHR. We found no significant differences between the aortae of our LBP-SHR and SHR in their responses to 10^{-7}–10^{-5} M NA.

The collagenase-treated aortae contracted to histamine in a dose-dependent manner (see Figure 3), which agrees with the observations of Van de Voorde & Leusen (1983). The magnitude of this contraction was also related to blood pressure, as the hypertensive animals were found to be significantly less responsive ($P<0.05$) to increasing doses of histamine. The degree of hyporesponsiveness was found to be more pronounced with increasing blood pressure as seen in the LRAS-LBP-SHR, indicating again that hypertension, *per se*, reduces the sensitivity of the arterial smooth muscle to directly acting vasoactive agents.

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